ELSEVIER

European Journal of Pharmacology 403 (2000) 163–167

ejp

www.elsevier.nl/locate/ejphar

Short communication

Effects of repeated administration of baclofen on transient lower esophageal sphincter relaxation in the dog

Anders Lehmann *, Lena Hansson-Brändén, Lillevi Kärrberg

Gastrointestinal Pharmacology, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

Received 5 July 2000; accepted 21 July 2000

Abstract

The metabotropic γ-aminobutyric acid (GABA) receptor ($GABA_B$ receptor) agonist baclofen inhibits transient lower esophageal sphincter relaxation in dogs, ferrets, and humans. Since transient lower esophageal sphincter relaxations are the major cause of gastroesophageal reflux, $GABA_B$ receptor agonists may have a therapeutic value in the treatment of gastroesophageal reflux disease. However, repeated stimulation of the $GABA_B$ receptor may induce receptor desensitization which, depending on the magnitude, would limit the therapeutic effect. The aim of the present study was to follow the effects of baclofen on transient lower esophageal sphincter relaxation in the dog after repeated administration. The effect of 7 μmol/kg baclofen b.i.d. (given intragastrically) on transient lower esophageal sphincter relaxation and related parameters was determined in four dogs. Transient lower esophageal sphincter relaxations stimulated by infusion of liquid nutrient and insufflation of air were quantified after placebo and then after the 1st, 13th, and 27th dose. Baclofen reduced the number of transient lower esophageal sphincter relaxations without affecting their duration, and the latency to the first transient lower esophageal sphincter relaxation was prolonged. Basal sphincter pressure was unaffected by baclofen, and the number of reflux episodes and esophageal acid exposure decreased. There was a statistically insignificant numerical decrease (approximately 30%) in the effect of baclofen on transient lower esophageal sphincter relaxation after the seventh dose but this was not further accentuated after the 27th dose. The effect on latency was also reduced with repeated dosing, but again, the effects after the 1st, 13th, and 27th doses were not statistically significant. The attenuation of acid exposure and reflux episodes was unaltered after repeated dosing. Three of the dogs greatly reduced their food intake within the first 2–3 days but this side effect was resolved subsequently. It is concluded that repeated dosing of baclofen leads to mild tolerance development in terms of the effects on transient lower esophageal sphincter relaxation, but that the tolerance is much less pronounced than that previously reported in other animal models.

Keywords: GABA (γ-aminobutyric acid); Esophageal sphincter relaxation, transient, lower; Baclofen; Tolerance

1. Introduction

The metabotropic γ-aminobutyric acid (GABA) receptor (the $GABA_B$ receptor) is activated by baclofen, the prototypic $GABA_B$ receptor agonist. Stimulation of the $GABA_B$ receptor produces several intracellular effects mediated partly by inhibition of adenylate cyclase (Bowery and Enna, 2000). In addition, inwardly rectifying K^+ channels are opened, and voltage dependent Ca^{2+} channels are closed. $GABA_B$ receptors are distributed throughout the central nervous system and in various peripheral organs. In nervous tissue, $GABA_B$ receptors may be present presynaptically to reduce evoked release of transmitters such as glutamate, substance P, and GABA (Bowery and Enna, 2000). $GABA_B$ receptors are also expressed postsynaptically where they mediate long-lasting inhibition.

Baclofen is used clinically mainly in the treatment of spasticity but it is also effective in conditions in which the vagus may be involved such as persistent hiccups (Ramirez and Graham, 1992) and coughing (Dicpinigaitis and Dobkin, 1997). Several reports have recently shown that in humans (Lidums et al., 2000), dogs (Lehmann et al., 1999), and ferrets (Blackshaw et al., 1999), baclofen suppresses yet another vagally dependent phenomenon, tran-

* Corresponding author. Tel.: +46-31-776-1945; fax: +46-31-776-3747.

E-mail address: anders.lehmann@astrazeneca.com (A. Lehmann).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00528-8

sient lower esophageal sphincter relaxations. Since these relaxations represent the major motility event underlying gastroesophageal reflux (Mittal et al., 1995), $GABA_B$ receptor stimulation may offer a novel approach to the treatment of gastroesophageal reflux disease that would be better targeted towards the cause of reflux compared with existing therapies.

As is commonly seen for other G protein coupled receptors, repeated pharmacological activation of the $GABA_B$ receptor is often associated with a reduced effect. With regard to attenuation of the effect of baclofen, the phenomenon is not related to altered pharmacokinetics which are not affected by repeated dosing (Faigle and Keberle, 1972). The magnitude of the desensitization of any given response cannot be predicted since it has been shown to differ between models. For example, the analgesic response to baclofen in mice (Vaught et al., 1985; Malcangio et al., 1992) and rats (Enna et al., 1998) is almost abolished after only a few doses of baclofen. Moreover, the ataxic/sedative effects of baclofen are obliterated after daily administration of baclofen for 5 days (Enna et al., 1998). However, repeated dosing of baclofen only produces a partial tolerance development with respect to hypothermia in the mouse (Gray et al., 1987). Whether or not tolerance will be present may not only be dependent on the variable studied but it may also depend on the $GABA_B$ receptor agonist used (Enna et al., 1998). In some (Kroin et al., 1993; Malcangio et al., 1993) but not all (Pratt and Bowery, 1993) studies, chronic administration of baclofen was accompanied by a diminished number of $GABA_B$ receptor binding sites. However, the relative importance of reduced density and/or affinity of receptors for ligands, compared with downregulation of $GABA_B$ receptor signalling due to other events, is unknown.

Tolerance development does occur but is not a major problem with regard to symptomatic treatment of spasticity with baclofen (Akman et al., 1993). Unexpectedly, the antitussive effect of baclofen in humans is only observed after repeated dosing (Dicpinigaitis and Dobkin, 1997; Dicpinigaitis et al., 1997) indicating that reactive changes to repeated administration of baclofen may have therapeutic effects. Whether $GABA_B$ receptor agonism can become a viable concept in the treatment of reflux disease depends to some extent on the degree of tolerance development. In the present study, the effects of baclofen given daily for 14 days on transient lower esophageal sphincter relaxation and related parameters were assessed in conscious dogs.

2. Materials and methods

2.1. General

Three adult Labrador retrievers and one beagle (all males) were accustomed to rest in a Pavlov sling. Mucosa-to-skin esophagostomies were made in the following way. General anesthesia was induced with propofol and maintained with isoflurane. A gastric tube introducer was inserted into the esophagus and the esophageal wall lifted towards the skin of the mid neck region. An incision was made through the skin and sternocephalicus muscle, medial to the external jugular vein and directly above the hollow end of the introducer. After the muscular layers of the esophagus were sutured to the sternocephalicus muscle, a 15-mm incision was made through the esophageal mucosa. The mucosa was then anchored to the skin with a row of single interrupted sutures. Skin sutures were removed 1 week later, and total recovery time after surgery was 1 month. The dogs were exposed to various drugs in other experiments, but no drugs were given at least 2 weeks before the present study. The experiments were approved by the Ethics Committee for Animal Experiments of the Gothenburg region.

2.2. Motility measurement

The methodology employed has been published previously (Lehmann et al., 1999). Briefly, a multilumen sleeve/sidehole assembly (Dentsleeve, Wayville, South Australia) was introduced through the ostomy to measure gastric, lower esophageal sphincter, and esophageal pressures after a 17 h fast with water given ad libitum. The assembly was perfused with water using a low-compliance manometric perfusion pump (Dentsleeve). An air-perfused tube was passed in the oral direction to measure swallows, and an antimony electrode monitored pH, 3 cm above the lower esophageal sphincter. All signals were amplified and acquired on a personal computer at 10 Hz.

When a baseline measurement free from fasting gastric/lower esophageal sphincter phase III motor activity had been obtained, placebo (0.9% NaCl) or baclofen dissolved in 0.9% NaCl was administered intragastrically (2 ml/kg) through one of the channels of the assembly. Thirty minutes after the administration, a nutrient meal (10% peptone, 5% D-glucose, 5% Intralipid, pH adjusted to 3.0 with HCl) was infused by means of a peristaltic pump into the stomach through the central lumen of the assembly at 100 ml/min to a final volume of 30 ml/kg. Immediately following the meal, air was insufflated at 40 ml/min. The experimental time from start of nutrient infusion to end of air insufflation was 45 min. The procedure has been validated as a reliable means of triggering transient lower esophageal sphincter relaxation (Lehmann et al., 1999).

Details on the definition and calculation of transient lower esophageal sphincter relaxation and most other motility parameters (e.g. duration of transient lower esophageal sphincter relaxation, basal lower esophageal sphincter pressure) have been given previously (Lehmann et al., 1999). In brief, transient lower esophageal sphincter relaxation were defined as a decrease in lower esophageal

sphincter pressure (with reference to intragastric pressure) at a rate of >1 mmHg/s. The relaxation should not be preceded by a pharyngeal signal <2 s before its onset in which case the relaxation was classified as swallow-induced. The pressure difference between the lower esophageal sphincter and the stomach should be less than 2 mmHg, and the duration of the complete relaxation longer than 1 s. Although latency from start of nutrient infusion to first transient lower esophageal sphincter relaxation may not have any clinical relevance, it was determined as an additional parameter reflecting changes in the triggering of transient lower esophageal sphincter relaxation. Basal lower esophageal sphincter pressure was defined as the average pressure difference between the sleeve and the intragastric pressure during the 45 min period. Periods of motility, defined as 10 s before and 20 s after a pressure increase >10 mmHg at the sidehole located 13 cm above the sleeve midpoint, were excluded from the analysis. This definition effectively excluded all swallow- and transient lower esophageal sphincter relaxation-related lower esophageal sphincter pressure changes and usually included 70% or more of the 45-min period.

Acid exposure was expressed as the percentage of the 45 min period during which intraesophageal pH was less than 4. Reflux episodes were defined as a drop in pH >1 unit in <5 s; pH should decrease below 5 and the average pH in the 15 s period following nadir should not increase more than 1 unit.

2.3. Chemicals

Baclofen was from RBI (Natick, MA), peptone from Boule Diagnostics (Huddinge, Sweden), D-glucose from Kebo (Spånga, Sweden) and Intralipid from Pharmacia&Upjohn (Stockholm, Sweden).

2.4. Calculations and statistical analysis

Each dog served as its own control, and the mean value of the five most recent placebo-controlled experiments was used. All parameters were calculated with regard to the 45-min period between the start of nutrient infusion to the discontinuation of air insufflation. The statistical analysis consisted of sequential comparison using Student's *t*-test between the results obtained in placebo-controlled experiments with those obtained after the 1st, 13th, and 27th dose of baclofen. To compensate for the fact that multiple comparisons were done, the post-hoc sequentially rejective multiple test (Holm, 1979) was employed.

3. Results

In agreement with earlier results, there was a significant decrease in the number of transient lower esophageal

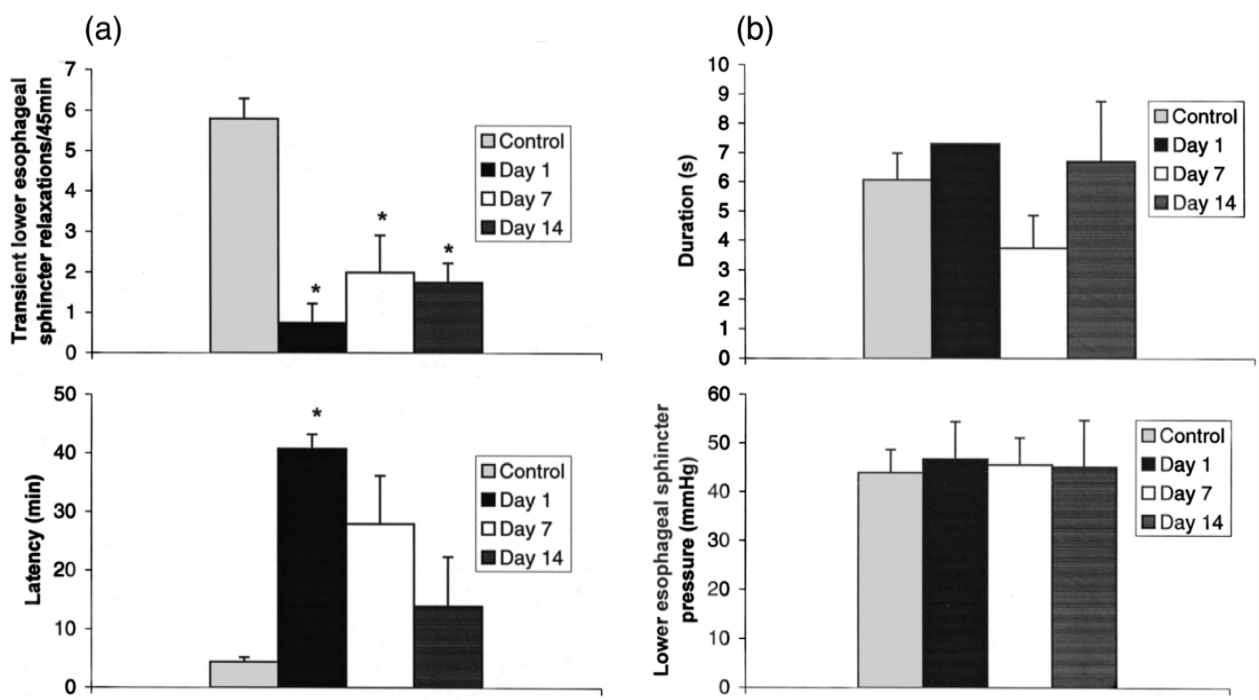

Fig. 1. Effects of repeated administration of baclofen on incidence of transient lower esophageal sphincter relaxation, latency to first transient lower esophageal sphincter relaxation, duration of transient lower esophageal sphincter relaxation and basal lower esophageal sphincter pressure. Baclofen was given intragastrically twice daily, and the effect was measured after the first dose on days 1, 7, and 14 of treatment. * $P<0.05$ compared with control (Student's *t*-test with Holm correction for multiple comparisons). There were no statistically significant differences between the effects of baclofen on day 1 compared with day 7 or 14.

sphincter relaxations after the first dose of baclofen (Fig. 1). Although there was a nominal reduction in the inhibitory effect of baclofen after the 13th and 27th dose, this could not be verified statistically. The inhibitory effects of baclofen were also reflected by a marked prolongation in the latency to the first transient lower esophageal sphincter relaxation from the start of nutrient infusion (Fig. 1). There was a tendency for this effect to decline with time, and only the effect of the first dose of baclofen was significantly different from control values. The duration of transient lower esophageal sphincter relaxations was unaffected in accordance with earlier results (Fig. 1). Further, basal lower esophageal sphincter pressure remained at control levels after the first dose and this lack of an effect of baclofen was also observed after repeated dosing (Fig. 1).

About half of the transient lower esophageal sphincter relaxations were accompanied by reflux detected pH-metrically. This proportion was not altered by baclofen (data not shown). The reduction in the number of reflux episodes caused by baclofen remained stable during repeated dosing (Fig. 2). In addition, esophageal acid exposure was significantly diminished by baclofen, and this effect was maintained during the course of the study (Fig. 2).

Food intake was normal in the beagle throughout the experimental period. One of the Labrador retrievers refused to eat on the 2nd day of dosing, but there was no need to discontinue the baclofen treatment. The two other Labradors did not eat at all on the 2nd and 3rd day, and the eighth and ninth doses of baclofen were therefore not administered. However, all subsequent doses could be given, and the dogs resumed eating. The cause of anorexia was not investigated, but one of the dogs that refused to eat vomited once, so nausea was considered a possible explanation. Apart from the change in feeding habits, none of the dogs showed any signs of side effects of the treatment.

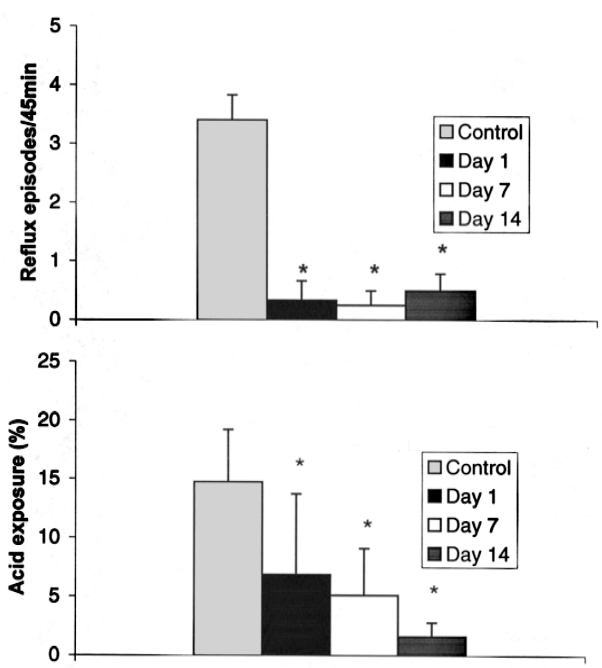

Fig. 2. Effects of daily dosing of baclofen b.i.d. for 14 days on the incidence of reflux episodes and esophageal acid exposure. $^{*}P < 0.05$ compared with control (Student's *t*-test with Holm correction for multiple comparisons). There were no statistically significant differences between the effects of baclofen on day 1 compared with day 7 or 14.

4. Discussion

The results obtained with the first dose of baclofen are in good agreement with our earlier data (Lehmann et al., 1999). Thus, baclofen greatly reduced the number of transient lower esophageal sphincter relaxations without affecting their morphology or basal pressure of the lower esophageal sphincter. In parallel, the latency to the first transient lower esophageal sphincter relaxation after the start of nutrient infusion and air insufflation was markedly prolonged, an effect that has been noted previously. In addition, the number of reflux episodes decreased more or less in parallel with transient lower esophageal sphincter relaxation incidence which was anticipated inasmuch as transient lower esophageal sphincter relaxations account for essentially all reflux in healthy dogs.

Since gastroesophageal reflux disease is a chronic disease, any drug with potential therapeutic utility has to be effective when given on a chronic basis. The present work suggests that the $GABA_B$ receptor agonist baclofen may fulfill this criterion. There was a nominal, non-significant partial loss of effect on transient lower esophageal sphincter relaxation after the seventh dose, but this was minor and it was not accentuated further after another week of treatment.

It can be argued that the experimental period was too short to effectively reveal tolerance development. For example, tolerance to the antispastic effect of baclofen in man may develop over a time course of several months (Akman et al., 1993). However, in most well-controlled preclinical studies, tolerance was shown to occur within a few days (Vaught et al., 1985; Gray et al., 1987; Enna et al., 1998). This agrees with our findings that hypothermic effect of baclofen in rats disappears completely after 7 days of dosing (unpublished). The time period studied in the current experiments must therefore be considered to be of sufficient duration for tolerance to develop.

A high dose of baclofen was chosen in order to increase the probability of producing tolerance. In retrospect, dosing with 7 μmol/kg b.i.d. may be considered redundant since the duration of effect of baclofen on transient lower esophageal sphincter relaxation has been shown to be unexpectedly long. The plasma half-life of the drug in the dog is 4 h, but the inhibition of transient lower esophageal sphincter relaxation persists unchanged for at least 5 h

(Lehmann et al., 1999) and more recent data suggest that there is still a significant effect 12 h after administration (own unpublished results). This contrasts the duration of antispastic effect in patients which necessitates t.i.d. dosing.

It was quite clear that in the animals which ate less or refused to eat initially after a few doses of baclofen, the appetite returned subsequently. Therefore, the anorexic effect showed obvious signs of tolerance development. The cause of this effect is unclear but appeared to be related to nausea, a well-known side effect of baclofen. Baclofen probably inhibits transient lower esophageal sphincter relaxation by acting on vagal afferents (Page and Blackshaw, 1999), and since they also are important for gastric function, the anorexic effect may be due to changes in gastric motility (gastric emptying, adaptive relaxation, etc.). However, we have failed to find a consistent effect of baclofen on emptying of liquid nutrients from the canine stomach (unpublished).

In summary, the present work indicates that $GABA_B$ receptor stimulation may be a useful concept in the treatment of gastroesophageal reflux disease, and that tolerance development may not be a limiting problem. However, baclofen itself may not be a useful drug in this regard since its side-effect profile is troublesome. Given the observation that development of tolerance to $GABA_B$ receptor agonists may be specific to the compound studied (Enna et al., 1998), any new $GABA_B$ receptor agonist that may be a candidate for treatment of gastroesophageal reflux disease should be tested in this regard.

References

Akman, M.N., Loubser, P.G., Donovan, W.H., O'Neill, M.E., Rossi, C.D., 1993. Intrathecal baclofen: does tolerance occur? Paraplegia 31, 516–520.

Blackshaw, L.A., Staunton, E., Lehmann, A., Dent, J., 1999. Inhibition of transient lower esophageal sphincter relaxations and reflux in ferrets by GABA receptor agonists. Am. J. Physiol. 277, G867–G874.

Bowery, N.G., Enna, S.J., 2000. γ-Aminobutyric $acid_B$ receptors: first of the functional metabotropic heterodimers. J. Pharmacol. Exp. Ther. 292, 2–7.

Dicpinigaitis, P.V., Dobkin, J.B., 1997. Antitussive effect of the GABA-agonist baclofen. Clin. Invest. 111, 996–999.

Dicpinigaitis, P.V., Dobkin, J.B., Rauf, K., 1997. Comparison of the antitussive effects of codeine and the GABA-agonist baclofen. Clin. Drug Invest. 14, 326–329.

Enna, S.J., Harstad, E.B., McCarson, K.E., 1998. Regulation of neurokinin-1 receptors expression by $GABA_B$ receptor agonists. Life Sci. 62, 1525–1530.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.

Faigle, J.W, Keberle, H., 1972. The chemistry and kinetics of Lioresal. Postgrad. Med. J. 48 (Suppl. 5), 9–13.

Gray, J.A., Goodwin, G.M., Heal, D.J., Green, A.R., 1987. Hypothermia induced by baclofen, a possible index of $GABA_B$ receptor function in mice, is enhanced by antidepressant drugs and ECS. Br. J. Pharmacol. 92, 863–870.

Kroin, J.S., Bianchi, G.D., Penn, R.D., 1993. Intrathecal baclofen down-regulates $GABA_B$ receptors in rat substantia gelatinosa. J. Neurosurg. 79, 544–549.

Lehmann, A., Antonsson, M., Bremner-Danielsen, M., Flärdh, M., Hansson-Brändén, L., Kärrberg, L., 1999. Activation of the $GABA_B$ receptor inhibits transient lower esophageal sphincter relaxations in dogs. Gastroenterology 117, 1147–1154.

Lidums, I., Lehmann, A., Checklin, H., Dent, J., Holloway, R.H., 2000. Control of transient lower esophageal sphincter relaxations and reflux by the $GABA_B$ agonist baclofen in normal subjects. Gastroenterology 118, 7–13.

Malcangio, M., Malmberg-Aiello, P., Giotti, A., Ghelardini, C., Bartolini, A., 1992. Desensitization of $GABA_B$ receptors and antagonism by CGP 35348, prevent bicuculline- and picrotoxin-induced antinociception. Neuropharmacology 31, 783–791.

Malcangio, M., Da Silva, H., Bowery, N.G., 1993. Plasticity of $GABA_B$ receptor in rat spinal cord detected by autoradiography. Eur. J. Pharmacol. 250, 153–156.

Mittal, R.K., Holloway, R.H., Penagini, R., Blackshaw, L.A., Dent, J., 1995. Transient lower esophageal sphincter relaxation. Gastroenterology 109, 601–610.

Page, A.J., Blackshaw, L.A., 1999. $GABA_B$ receptors inhibit mechanosensitivity of primary afferent endings. J. Neurosci. 19, 8597–8602.

Pratt, G.D., Bowery, N.G., 1993. Repeated administration of desipramine and a $GABA_B$ receptor antagonist, CGP 36742, discretely up-regulates $GABA_B$ receptor binding sites in rat frontal cortex. Br. J. Pharmacol. 110, 724–735.

Ramirez, F.C., Graham, D.Y., 1992. Treatment of intractable hiccup with baclofen: results of a double-blind randomized, controlled, cross-over study. Am. J. Gastroenterol. 87, 1789–1791.

Vaught, J.L., Pelley, K., Costa, L.G., Setler, P., Enna, S.J., 1985. A comparison of the antinociceptive responses to the GABA-receptor agonists THIP and baclofen. Neuropharmacology 24, 211–216.